

Attorney Docket No. P66034US0
Application No. 09/674,277

Amendments to the claims:

This listing of claims replaces all prior versions, and listings, of claims in the application.

Listing of claims:

Claims 1-19 (cancelled).

20 (currently amended): ~~A~~ An isolated nucleic acid consisting of nucleotide sequence SEQ ID NO: 1, or nucleic acid sequence SEQ ID NO. 2, their complementary sequences, a fragment of SEQ ID NO: 2 or a sequence derived from SEQ ID NO: 2 by mutation, ~~insertion~~, deletion, and/or substitution of one or more bases, said derived sequence hybridizing under high stringency conditions with sequence SEQ ID NO: 2, wherein each of said fragment and said derived sequence specifically detects enterohaemorrhagic *Escherichia coli* (EHECs).

21 (currently amended): An isolated nucleic acid consisting of a fragment of nucleotide sequence SEQ ID NO: 1, or a sequence derived from SEQ ID NO: 1 by mutation, ~~insertion~~, deletion, and/or substitution of one or more bases, said derived sequence hybridizing under high stringency conditions with sequence SEQ ID NO:1, said fragment or derived sequence containing a nucleotide chain of SEQ ID NO: 1 resulting from stable combination of at least a portion of insertion sequence

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IS91 and at least a portion of gene sequence *katP*, wherein each of said fragment and said derived sequence specifically detects enterohaemorrhagic *Escherichia coli* (EHECs).

22 (previously presented): The isolated nucleic acid according to claim 21, including at least nucleotide chain 400 to 407 of SEQ ID NO: 1.

23 (previously presented): The isolated nucleic acid according to claim 20, each of said fragment and derived sequence being a nucleotide chain of at least 8 consecutive nucleotides of SEQ ID NO: 2.

24 (currently amended): The isolated nucleic acid according to claim ~~22~~ 21 selected from the group consisting of:

SEQ ID NO: 10 : 5'-AAGGGGTTCCAAGCCGCAACTGACGA-3'

SEQ ID NO: 11 : 5'-TAAGGGGTTCCAAGCCGCAACTGACG-3' ¹⁰

SEQ ID NO: 12 : 5'-CTCAACGGCATCGTCAGTTGCGGCTTGGAAC-3'

SEQ ID NO: 13 : 5'-AGCACTCAACGGCATCGTCAGTTGCGGCTTG-3'

SEQ ID NO: 18 : 5'-GGCATCGTCAGTTG-3'

SEQ ID NO: 19 : 5'-CGGCATCGTCAGTTGC-3' and

SEQ ID NO: 20 : 5'-ACGGCATCGTCAGTTGCG-3'

~~SEQ ID NO: 21 : 5'-CCACCTGAACGATAAGCGGAAC-3'~~

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~~SEQ ID NO: 22 : 5'-CACCTTCCTTCCATCCTCAGAC-3'~~

~~SEQ ID NO: 23 : 5'-ATCCCAGCGCGCTCCAGCTG-3'~~

~~SEQ ID NO: 24 : 5'-ACCCATGATGGCGCATCTGATG-3'~~

~~SEQ ID NO: 25 : 5'-ACGTTCTGGTCTTACGGGTGATGTAGGTTTT-3'~~

~~SEQ ID NO: 26 : 5'-TAGTGAAAGCGGTGACAGCATATCAGACGGCT-3'~~ and

~~SEQ ID NO: 27 : 5'-GTGAGATAGGCACAACAATGA-3'~~

25 (currently amended): The isolated nucleic acid according to claim ~~23~~ 20 selected from the group consisting of:

~~SEQ ID NO: 10 : 5'-AAGGGGTTCCAAGCGGCAACTGACGA-3'~~

~~SEQ ID NO: 11 : 5'-TAAGGGGTTCCAAGCGGCAACTGACG-3'~~ ~~10~~

~~SEQ ID NO: 12 : 5'-CTCAAAGGGCATEGTCAGTTGCGGCTTGGAAAC-3'~~

~~SEQ ID NO: 13 : 5'-AGCACTCAAAGGGCATEGTCAGTTGCGGCTTG-3'~~

~~SEQ ID NO: 18 : 5'-GGCATCGTCAGTTG-3'~~

~~SEQ ID NO: 19 : 5'-CGGCATCGTCAGTTGC-3'~~

~~SEQ ID NO: 20 : 5'-ACGGCATEGTCAGTTGCG-3'~~

SEQ ID NO: 21 : 5'-CCACCTGAACGATAAGCGGAAC-3'

SEQ ID NO: 22 : 5'-CACCTTCCTTCCATCCTCAGAC-3'

SEQ ID NO: 23 : 5'-ATCCCAGCGCGCTCCAGCTG-3'

SEQ ID NO: 24 : 5'-ACCCATGATGGCGCATCTGATG-3'

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SEQ ID NO: 25 : 5'-ACGTTCTGGTCTTACGGGTGATGTAGGTTTT-3'

SEQ ID NO: 26 : 5'-TAGTGAAGCGGTGACAGCATATCAGACGGCT-3' and

SEQ ID NO: 27 : 5'-GTGAGATAGGCACAACAATGA-3'.

Claim 26 (cancelled).

27 (previously presented): Plasmid pDF3 deposited at Collection Nationale de Cultures de Microorganismes under number I-1999, on 26 March 1998.

28 (previously presented): Plasmid pDF4 deposited at Collection Nationale de Cultures de Microorganismes under number I-2000, on 26 March 1998.

29 (previously presented): A host cell comprising the plasmid according to claim 27.

30 (previously presented): A host cell comprising the plasmid according to claim 28.

31 (previously presented): A method for detecting a enterohaemorrhagic Escherichia Coli (EHEC) in a sample, said method comprising detecting a nucleotide chain of sequence SEQ ID NO: 2 by contacting the sample with the isolated nucleic acid as defined in claim 23, the presence of said nucleotide chain being indicative of the presence of a EHEC.

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32 (previously presented): The method according to claim 31, wherein said isolated nucleic acid is selected from the group consisting of SEQ ID NOS: 22-27.

33 (previously presented): The method according to claim 31, wherein said isolated nucleic acid functions as a primer and/or a probe.

34 (previously presented): The method according to claim 31, wherein said isolated nucleic acid is detectably labeled.

35 (previously presented): The method according to claim 31, wherein said isolated nucleic acid is immobilized on a support.

36 (previously presented): The method according to claim 31, comprising the steps of:

- (a) bringing the sample into contact with a pair of oligonucleotide primers selected from the group consisting of SEQ ID NO: 21-27, the nucleic acid contained in the sample having been, where appropriate, made accessible to the said primers at the target tested for,
- (b) amplifying the nucleic sequence flanked by the pair of primers,
- (c) verifying presence of the amplified product by the use of at least one probe specific for the amplified product.

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37 (previously presented): The method according to claim 36, wherein step (c) comprises the substeps of:

- (c₁) denaturation of the amplified sequences by physical or chemical means,
- (c₂) bringing a solution containing the denatured amplified sequences of step (c₁) into contact with at least one capture probe and at least one detection probe, optionally labeled, for a period of time sufficient to allow hybridization of the capture and detection probes with the same strand of the denatured amplified sequences,
- (c₃) washing at least once in order to remove unreacted denatured amplified sequences,
- (c₄) visualization of the detection probes hybridized with the denatured amplified sequences.

38 (previously presented): The method according to claim 37, in which the capture probe is attached to the surface of a well of a microtiter plate.

39 (previously presented): The method according to claim 37, in which the detection probe is labeled with peroxidase.

40 (previously presented): The method according to claim 39, wherein detection of activity of the peroxidase linked to a hybridized detection probe is carried out by colorimetric reaction, in the presence of a chromogenic substrate, comprising the steps of:

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- addition of the chromogenic substrate to the wells of the microtiter plate containing the solution of denatured amplified sequences,
- incubation, in the dark, for a sufficient period of time to allow peroxidase color to develop,
- stopping the hybridization by addition of a blocking solution,
- determination of the optical density at an appropriate wavelength.

41 (previously presented): The method according to claim 37, using the following oligonucleotides:

- sequences SEQ ID NO: 21 and SEQ ID NO: 22, as primers for the amplification,
- sequence SEQ ID NO: 25, as capture probe,
- sequence SEQ ID NO: 27, as detection probe.

42 (previously presented): A method for detecting *E. coli* 0157:H7 in a sample, which method comprises detecting a nucleotide chain resulting from the stable combination of at least a portion of the insertion sequence *IS91* and at least a portion of the sequence of the *katP* gene with the aid of an isolated nucleic acid comprising at least eight consecutive nucleotides of the sequence SEQ ID NO: 1, the sequence complementary thereto, or of a sequence derived therefrom, differing by mutation, insertion, deletion and/or substitution of one or more bases and hybridizing under high stringency conditions with the sequence SEQ ID NO: 1, the presence of said nucleotide chain being indicative of the presence of *E. coli* 0157:H7.

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43 (previously presented): The method according to claim 42, wherein the sequence of said isolated nucleic acid is selected from the group consisting of sequence SEQ ID NO: 3 - SEQ ID NO: 20.

44 (previously presented): The method according to claim 42, which further comprises detecting a nucleotide chain of sequence SEQ ID NO: 2 with the aid of a second isolated nucleic acid, the second isolated nucleic acid being a chain of at least eight consecutive nucleotides of SEQ ID NO: 2, the presence of said nucleotide chain being indicative of the presence of a EHEC.

45 (currently amended): The method according to claim 44, wherein the sequence of said second isolated nucleic acid is selected from the group consisting of:

SEQ ID NO: 21 : 5'-CCACCTGAACGATAAGCGGAAC-3'

SEQ ID NO: 22 : 5'-CACCTTCCTTCCATCCTCAGAC-3' 5

SEQ ID NO: 23 : 5'-ATCCCAGCGCGCTCCAGCTG-3'

SEQ ID NO: 24 : 5'-ACCCATGATGGCGCATCTGATG-3'

SEQ ID NO: 25 : 5'-ACGTTCTGGTCTTACGGGTGATGTAGGTTTT-3'

SEQ ID NO: 26 : 5'-TAGTGAAGCGGTGACAGCATATCAGACGGCT-3' and

SEQ ID NO: 27 : 5'-GTGAGATAGGCACAACAATGA-3'.

46 (previously presented): The method according to claim 42, wherein said isolated nucleic acid is used as a primer and/or a probe.

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47 (previously presented): The method according to claim 42, wherein said isolated nucleic acid is detectably labeled.

48 (previously presented): The method according to claim 42, wherein said isolated nucleic acid is immobilized on a support.

49 (previously presented): The method according to claim 42, comprising the steps of:

- (a) bringing the sample into contact with a pair of oligonucleotide primers selected from the group consisting of sequences SEQ ID NO: 3 - SEQ ID NO: 20, such that the nucleic acid in the sample is flanked by the pair of nucleotide primers, the nucleic acid contained in the sample having been, where appropriate, made accessible to the pair of oligonucleotide primers at the target tested for,
- (b) amplifying the nucleic acid sequence flanked by the pair of oligonucleotide primers to produce an amplified product,
- (c) verification of the presence of the amplified product by using at least one probe specific for the amplified product.

50 (previously presented): The method according to claim 49, according to which step (c) comprises the following substeps:

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- (c₁) denaturation of the amplified product sequences into fragments by physical or chemical means,
- (c₂) (i) bringing a solution containing the fragments of amplified product from step (c₁) into contact with at least one capture probe and at least one detection probe, optionally detectably labeled, the capture and detection probes being capable of hybridizing with the same fragment strand, when present, in the fragments of amplified product, and (ii) maintaining the contact for a period of time sufficient to allow the hybridization reaction,
- (c₃) washing at least once in order to remove unreacted fragments of amplified product,
- (c₄) visualization of hybridized detection probes.

51 (previously presented): The method according to claim 50, in which the capture probe is attached to the surface of a well of a microtiter plate.

52 (previously presented): The method according to claim 50, in which the detection probe is labeled with peroxidase.

53 (previously presented): The method according to claim 52, wherein detection of peroxidase activity is carried out by colorimetric reaction, in the presence of a chromogenic substrate, comprising the steps of:

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- addition of the chromogenic substrate to the wells of the microtiter plate containing the solution of denatured amplified sequences,
- incubation, in the dark, for a sufficient period of time to allow peroxidase color to develop,
- stopping the hybridization by addition of a blocking solution,
- determination of the optical density at an appropriate wavelength.

54 (previously presented): The method according to claim 50 wherein:

- the pair of oligonucleotide primers are sequences SEQ ID NO: 5 and SEQ ID NO: 6,
- the capture probe is sequence SEQ ID NO: 15,
- the detection probe is sequence SEQ ID NO: 18.

55 (currently amended): A kit for detecting EHECs, comprising:

- at least two oligonucleotides primers selected from the group consisting of:

SEQ ID NO: 21 : 5'-CCACCTGAACGATAAGCGGAAC-3'

SEQ ID NO: 22 : 5'-CACCTTCCTTCCATCCTCAGAC-3' 5

SEQ ID NO: 23 : 5'-ATCCCAGCGCGCTCCAGCTG-3'

SEQ ID NO: 24 : 5'-ACCCATGATGGCGCATCTGATG-3'

SEQ ID NO: 25 : 5'-ACGTTCTGGTCTTACGGGTGATGTAGGTTTT-3'

SEQ ID NO: 26 : 5'-TAGTGAAGCGGTGACAGCATATCAGACGGCT-3' and

SEQ ID NO: 27 : 5'-GTGAGATAGGCACAACAATGA-3'

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- optionally at least one oligonucleotide probe selected from the group consisting of:

SEQ ID NO: 21 : 5'-CCACCTGAACGATAAGCGGAAC-3'

SEQ ID NO: 22 : 5'-CACCTTCCTTCCATCCTCAGAC-3' 5

SEQ ID NO: 23 : 5'-ATCCCAGCGCGCTCCAGCTG-3'

SEQ ID NO: 24 : 5'-ACCCATGATGGCGCATCTGATG-3'

SEQ ID NO: 25 : 5'-ACGTTCTGGTCTTACGGGTGATGTAGGTTTT-3'

SEQ ID NO: 26 : 5'-TAGTGAAGCGGTGACAGCATATCAGACGGCT-3' and

SEQ ID NO: 27 : 5'-GTGAGATAGGCACAACAATGA-3'.

56 (previously presented): A kit for detecting EHECs according to claim 55, comprising:

- two oligonucleotides of sequences SEQ ID NO: 21 and SEQ ID NO: 22, as a pair of primers,
and
- two oligonucleotides of sequences SEQ ID NO: 25 and SEQ ID NO: 27, for detection.

57 (previously presented): A kit for detecting *E. coli* 0157:H7, comprising:

- at least two oligonucleotide primers selected from the group consisting of sequences SEQ ID NO: 3 - SEQ ID NO: 20; and, optionally,
- at least one oligonucleotide probe selected from the group consisting of sequences SEQ ID NO: 3 - SEQ ID NO: 20.

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58 (previously presented): A kit for detecting *E. coli* 0157 :H7 according to claim 57, comprising:

- two oligonucleotides of sequences SEQ ID NO: 5 and SEQ ID NO: 6, as a pair of primers,
- two oligonucleotide of sequences SEQ ID NO: 15 and SEQ ID NO: 18, for detection.

59 (previously presented): The method of claim 40, wherein the chromogenic substrate is tetramethylbenzidine (TMB).

60 (previously presented): The method of claim 53, wherein the chromogenic substrate is tetramethylbenzidine (TMB).